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NATIONAL DEPARTMENT OF DEFENSE SURVEILLANCE FOR CLINICAL GROUP A STREPTOCOCCAL ISOLATES, ANTIBIOTIC RESISTANCE, AND EMM GENE TYPES FROM 8 BASIC TRAINING MILITARY SITES

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National Department of Defense Surveillance for Clinical Group A Streptococcal Isolates, Antibiotic Resistance, and *emm* Gene Types From 8 Basic Training Military Sites

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Abstract

Active surveillance for group A streptococci (GAS) was conducted among military trainees with pharyngitis at 8 U.S. military basic training sites between January 1998 and December 2001. Antibiotic resistance and *emm* gene type distribution were assessed for 692 GAS isolates. Antibiotic susceptibility testing revealed 100% sensitivity to penicillin, levofloxacin and vancomycin. Forty-four isolates (6.4%) were resistant to erythromycin, 38 (5.5%) resistant to tetracycline, 22 (3.2%) resistant to clindamycin, and 14 isolates (2.0%) showed multidrug resistance. The most prevalent genotypes were *emm29* (18.0%), *emm3* (15.2%), *emm6* (13.5%), *emm44/61* (9.1%), *emm2* (7.3%), *emm75* (6.4%), and *emm1* (4.8%). An association was found among distinct *emm* types and geographic location. Erythromycin resistance was strongly associated with *emm*75 and *emm*29 isolates ($p < 0.001$). Continued monitoring of antibiotic susceptibility and genetic epidemiology of GAS isolates is important for directing appropriate prevention and treatment strategies among U.S. military populations.

Introduction

Group A streptococci (GAS; *Streptococcus pyogenes*) are responsible for a variety of illnesses that affect humans. GAS diseases range from common pharyngitis, epiglottitis, and pneumonia to devastating manifestations of acute rheumatic fever (ARF), necrotizing fasciitis, sepsis, and streptococcal toxic shock syndrome [1]. In the 1980s both U.S. civilian and military personnel experienced numerous outbreaks of ARF, the first for the U.S. military in over 20 years [2-4]. Epidemiological data suggest that these epidemics may have been at least partially due to emergent, likely more virulent GAS strains [5-7]. Historically, military recruits are at high risk for streptococcal illness outbreaks due to crowded living conditions, and numerous stressors [8-10].

Currently, the U.S. military implements mass penicillin prophylaxis among its training populations with good success [9, 11, 12]. Year-round benzathine penicillin G injections are given at some recruit training sites. At other sites, implementation of penicillin vaccination among recruits is seasonal and/or dynamic and modified depending on local surveillance indicators. Depending upon the training site, penicillin-allergic individuals receive no antibiotic, or a substitute antibiotic, such as erythromycin [13]. Despite this exhaustive coverage, GAS infections and outbreaks continue to occur.

With antibiotic resistance among bacterial pathogens on the rise, in 1998 we established GAS surveillance at 8 military training facilities throughout the United States [14]. Our primary objective was to monitor antibiotic susceptibility patterns and the molecular epidemiology of this militarily important pathogen.

Methods

Demographic Data

Between January 1998 and December 2001, a systematic sample of noninvasive GAS isolates was collected from pharyngeal cultures of symptomatic military trainees as a part of standard medical care. Eight U.S. military basic training sites participated: Naval Recruit Training Center, Great Lakes, Illinois; Marine Corps Recruit Depot, San Diego, California; Marine Corps Recruit Depot, Parris Island, South Carolina; Army Basic Training Centers in Fort Jackson, South Carolina, Fort Knox, Kentucky, Fort Leonard Wood, Missouri, and Fort Sill, Oklahoma; and the Air Force Basic Training Center at Lackland Air Force Base, Texas. The following demographic data were collected for each isolate: study identification number, last four digits of patient's social security number, specimen collection date, study site, age in years, and gender.

Susceptibility Testing

Study sites preserved *S. pyogenes* specimens in tryptic soy broth with 15% glycerol at –70°C until transport to the Naval Health Research Center, San Diego. Isolates were reconfirmed as GAS by colony morphology on 5% sheep blood agar plates, sensitivity to bacitracin, and a positive reaction to a latex agglutination test (Hardy Diagnostics, Santa Maria, CA). Antibiotic susceptibility testing was performed with E-test antimicrobial gradient strips (AB Biodisk, Piscataway, NJ) using National Committee for Clinical Laboratory Standards for minimum inhibitory concentration (MIC) interpretations and quality control ranges [15, 16]. GAS isolates were tested for resistance to penicillin, erythromycin, clindamycin, tetracycline, levofloxacin, and vancomycin. The plates were incubated for 18 to 24 hours at 36°C with 5% CO_2. Multiple drug resistance was defined as resistance to two or more antibiotics.

Molecular: *emm* Typing

The GAS isolates were *emm* typed using procedures adapted from the Centers for Disease Control and Prevention (CDC) and the World Health Organization [17, 18]. GAS isolates were grown in Todd-Hewitt broth overnight at 36°C in 5% CO_2. After centrifugation, growth was resuspended in sterile saline and incubated for 30 minutes at 60°C. Cell pellets were again suspended in a mixture of 10mM Tris, 1mM EDTA, pH8, 3000 units/ml of mutanolysin, and 30 mg/ml of hyaluronidase and incubated for 1 hour at 37°C, followed by 100°C for 10 minutes. DNA was saved after centrifugation and then used in a 100 ul PCR mixture with Primer1 and Primer2 [19]. PCR products were purified with QIAquick purification columns (Qiagen, Valencia, CA). Samples were amplified with Big Dye Terminator chemistry (Applied Biosystems, Foster City, CA), and analyzed by ABI Prism 3100 automated sequencer as described by manufacturer. DNA sequences were submitted to a blast-search (www.cdc.gov/ncidod/biotech/strep/strepblast.htm) for *emm* type determination. A 95% or greater homology with a reference *emm* gene sequence was required for assignment of *emm* type [17].

Statistical Analysis

Univariate analyses, including Pearson chi-square exact tests or Monte Carlo estimated exact tests, were initially performed to assess possible associations between demographic variables and antibiotic resistance or *emm* type. Variables associated with the outcome of interest (as characterized by p-value ≤ 0.15) were included in subsequent exact multivariable logistic regression model analyses. Using regression diagnostics, collinearity among variables was investigated. Additionally, predictors contributing more than their joint effects were investigated by introducing cross-product terms into the model to test for significance of interaction. The saturated models were reduced by a manual backward stepwise elimination approach. Final

models included only those variables independently associated with the outcome of interest with p-values ≤ 0.05. Using SAS® (Version 8.0, SAS Institute, Cary, NC), odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for variables associated with the outcome of interest.

Results

From January 1998 to December 2001, 692 GAS samples were received from the 8 military basic training sites. The largest percentage of isolates was collected during the fall season (36.4%). The majority of the samples came from males (85.9%). Eighty-four percent of the samples were from new military recruits ages 17–22 years (table 1).

Antibiotic Susceptibility

One hundred percent of isolates tested were susceptible to penicillin (MIC, <0.12 μg/ml), levofloxacin (MIC, <2.0 μg/ml), and vancomycin (MIC, <1.0μg/ml). Forty-four isolates (6.4 %) were resistant to erythromycin (MIC range, 0.5 – ≥1.0μg/ml), 38 (5.5%) were resistant to tetracycline (MIC range, 4.0 – ≥8.0μg/ml), 22 (3.2%) were resistant to clindamycin (MIC range 0.5 – ≥1.0μg/ml), and 14 (2.0%) showed partial or full resistance to two or more antibiotics.

Univariate analyses suggested that gender and geographic site were associated with antibiotic resistance. Multivariable modeling demonstrated that isolates from women were more likely to be resistant to clindamycin (OR = 7.3, 95% CI, 2.9-18.5) or tetracycline (OR = 2.2, 95% CI, 1.0-4.8). In addition, isolates from the Air Force site in Texas were much more likely to be resistant to erythromycin (OR = 25.1, 95% CI, 11.0-57.1) while isolates from Army sites in Kentucky and Missouri were more likely to be resistant to tetracycline (OR = 6.6, 95% CI, 2.1-21.8 and OR = 6.7, 95% CI, 2.6-17.7, respectively) (tables 2 and 3).

Surveillance for GAS antibiotic resistance revealed some temporal patterns, most notably with increased erythromycin resistance in 1999, and increased tetracycline resistance in 2001 (data not shown).

Molecular Typing

Six hundred and eighty-six isolates were *emm* typed (99.1%); over 30 different types were identified. The most prevalent were *emm29* (18.0%), *emm3* (15.2%), *emm6* (13.5%), *emm44/61* (9.1%), *emm2* (7.3%), *emm75* (6.4%), and *emm1* (4.8%). Heterogeneity of *emm* types was noted at each military site; however, univariate analyses demonstrated that training site was statistically associated with *emm* type (p-value <0.001, Pearson exact chi-square). The Navy's Illinois site had proportionally more *emm6* and *emm44/61*, 32.9% and 27.8%, respectively. Both *emm29* (43.2%) and *emm*3 (25.7%) types were more prevalent at the Marine site in South Carolina. The Air Force's site in Texas had a predominance of *emm75* among its GAS isolates (30.7%). Data were sparse for the Army sites, but *emm3*, *emm1*, *emm75*, and *emm27L/77* were found (table 4). Additionally, the prevalence of *emm* types varied from year to year (figure 1). In our study, *emm6* and *emm3* were prevalent in 1998 and 2001, but were infrequent in 1999-2000. Likewise, *emm29* and *emm44/61* were more prevalent in 2000-2001, but nearly absent in 1998-1999.

Univariate analysis also demonstrated an association between *emm* types and antibiotic resistance (data not shown). Erythromycin resistance was strongly associated with *emm29* ($p < 0.001$) and *emm75* isolates ($p < 0.001$), and less strongly associated with *emm3* ($p = 0.0028$), *emm6* ($p = 0.0092$), and *emm44/61* ($p = 0.0472$). Tetracycline resistance patterns were found associated with *emm29* ($p = 0.0176$), *emm3* ($p = 0.0112$), and *emm6* ($p = 0.0162$), while clindamycin resistance was associated with *emm29* ($p = 0.0382$) and *emm44/61* ($p = 0.035$).

Discussion

As a population with exposures potentially conducive to microbial spread, the U.S. military gives high priority to infection control programs [20-23]. Efforts to control Group A streptococcal infections are no exception. Recruit personnel arriving at basic training are often prophylactically treated with one or more injections of benzathine penicillin. Alternatively, for penicillin-allergic individuals, oral erythromycin regimens are administered to reduce GAS transmission. This prevention strategy has generally proved to be quite effective in minimizing effects of streptococcal disease [9-11, 13], although outbreaks still occasionally occur.

With the availability of rapid diagnostic tests, primary pharyngeal culture is becoming less common. Hospitals and clinics do not routinely perform susceptibility testing for GAS isolates they culture, leaving antibiotic resistance of GAS not well documented in the United States. Penicillin continues to be widely recognized as the drug of choice for GAS infections. Our surveillance demonstrates continued 100% penicillin susceptibility among noninvasive infections among military training populations.

Recent work on GAS by Martin and colleagues among school-age children in the United States found the emergence of erythromycin resistance similar to that seen in Asia and Europe [24-26]. Fortunately, erythromycin resistance among U.S. military training populations remains at a relatively low (6.4%) prevalence. It is possible that antibiotic failure for other prescribed antibiotics may be higher than generally recognized. We noted a prevalence of 5.5% tetracycline and 3.0% clindamycin resistance among our isolates. The development of antibiotic-resistant clones of GAS is a major concern for both military and civilian populations. In addition, our data suggest that antibiotic resistance among GAS isolates is not confined to a single geographic area.

Resistance was found at all military sites, with a high of 29.4% erythromycin resistance at the Air Force training site.

From the molecular perspective, several GAS virulence factors are suggested to be associated with more severe disease [27]. The M protein is one of these important virulence factors, offering GAS several mechanisms of defense against the human immune system, most notably the ability to evade phagocytosis. The variable 5' sequences of the *emm* gene, encoding for the M serotypes, have been associated with virulent strains of GAS [28-31]. Recent literature suggests that *emm*1 and *emm*3, for example, are among the virulent strains [32, 33]. The new *emm* gene typing method in contrast to the old M-serotyping methods that sometimes failed to identify 50% of isolates provides a more definitive molecular epidemiological tool for studying GAS isolates [17].

Among the *emm* types identified in the course of this surveillance, univariate analyses revealed that *emm75* had a statistically significant association with erythromycin resistance. This study was not able to assess the mechanism of resistance for these isolates, but other studies have suggested the presence of efflux mechanisms and resistance genes [34, 35]. Though there was high prevalence of erythromycin resistance among *emm75* isolates, there were several other *emm* types that were more prevalent in our surveillance.

Many common *emm* types reported by the CDC in its Active Bacterial Core Surveillance (ABCs) for GAS were found in our surveillance: *emm1*, *emm3*, *emm12*, *emm28*, and *emm89* (see table 4). Notably, the ABCs report monitors invasive disease, while our surveillance was of noninvasive cases. The concordance of *emm* types noted from invasive and noninvasive presentations highlights the concern that virulent strains of GAS may be circulating in noninvasive illnesses. One might hypothesize that failing to properly manage streptococcal infections could result in escalating invasive disease trends and mortality from such strains.

The heterogeneity of *emm* types observed in this study reflects the importance of considering multiple *emm* types in designing GAS vaccines. One could consider the idea of a geographical type-specific vaccine or possibly a year-to-year variation, much like the influenza vaccine is distributed each year. Since the M protein is currently described as a major virulence factor in GAS infections, a number of current vaccine constructs are composed of multivalent M protein sequences specific to particular diseases or geographic regions [36]. Our surveillance data may help to guide these constructs.

In conclusion, active surveillance for GAS isolates among U.S. military trainees with pharyngitis has revealed a significant prevalence of macrolide antibiotic resistance. The *emm* type distribution varied across military training sites, with *emm75* strongly correlated with erythromycin resistance at an Air Force base in Texas. Small outbreaks of antibiotic-resistant GAS may go unnoticed without proper surveillance methods, thus promoting further increased resistance due to improper utilization of antibiotics. The use of erythromycin in penicillin-allergic individuals may be reconsidered at sites with a background of increased macrolide resistance in GAS. Continued active surveillance, including antibiotic resistance and *emm* typing, is expected to provide critical information for GAS prevention and treatment strategies within the U.S. military. In addition, increased understanding of the epidemiology of GAS infections is important for the potential development and use of vaccines that may minimize streptococcal disease morbidity in both civilian and military populations.

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Table 1. Characteristics of military trainees who contributed Group A streptococcal isolates.

Variable	Total (N = 692)	Percent (%)	Population proportions* (%)
Gender†			
Male	592	85.9	81.4
Female	97	14.1	18.6
Age, y††			
17-18	196	28.6	55.9
19-20	281	41.0	24.4
21-22	99	14.5	9.5
$\geq$ 23	109	15.9	10.3
Season			
Summer (Jun-Aug)	73	10.6	33.0
Fall (Sep-Nov)	252	36.4	27.0
Winter (Dec-Feb)	161	23.3	20.0
Spring (Mar-May)	206	29.7	20.0
Training site			
Navy, Illinois	222	32.1	22.1
Marines, California	3	0.4	8.2
Marines, South Carolina	282	40.7	8.9
Army, South Carolina	4	0.6	18.6
Army, Kentucky	23	3.3	6.2
Army, Missouri	37	5.4	11.1
Army, Oklahoma	19	2.8	6.2
Air Force, Texas	102	14.7	18.6

*Relative proportions of military trainees at basic training sites in each of the given stratagem.
†Missing gender data from 3 patients.
††Missing age data from 7 patients .

Table 2. Factors associated with erythromycin resistance among GAS isolates: results of multivariable logistic regression modeling.

	Total isolates	Resistant isolates (%)	OR	95% CI
Gender				
Male*	592	34 (5.7)	--	--
Female	97	10 (10.3)	NSS	
Age, y[†]				
17-18*	196	13 (6.6)	--	--
19-20	281	20 (7.1)	NSS	
21-22	99	6 (6.1)	NSS	
> 22	109	5 (4.6)	NSS	
Season				
Summer (Jun-Aug)*	73	5 (6.9)	--	--
Fall (Sep-Nov)	252	7 (2.8)	NSS	
Winter (Dec-Feb)	161	10 (6.2)	1.5	(0.6–3.8)
Spring (Mar-May)	206	22 (10.7)	2.2	(1.1–4.9)
Training site				
Navy, Illinois*	222	7 (3.2)	--	--
Marines, California	3	0 (0.0)	NSS	
Marines, South Carolina	282	1 (0.4)	NSS	
Army, South Carolina	4	0 (0.0)	NSS	
Army, Kentucky	23	2 (8.7)	5.4	(1.1–27.3)
Army, Missouri	37	4(10.8)	7.1	(2.0–25.0)
Army, Oklahoma	19	0 (0.0)	NSS	
Air Force, Texas	102	30 (29.4)	25.1	(11.0–57.1)

NOTE. OR = odds ratio; CI = confidence interval; NSS = not statistically significant.
*Reference category for multivariable logistic regression.
[†]Variable not statistically significant at the univariate level; not included in multivariable model.

Table 3. Factors associated with tetracycline resistance in GAS isolates: results of multivariable logistic regression modeling.

	Total isolates	Resistant isolates (%)	OR	95% CI
Gender				
Male*	592	28 (4.7)	--	--
Female	97	10 (10.3)	2.2	(1.0–4.8)
Age, y[†]				
17-18*	196	14 (7.1)	--	--
19-20	281	11 (3.9)	NSS	
21-22	99	6 (6.1)	NSS	
> 22	109	6 (5.5)	NSS	
Season				
Summer (Jun-Aug)*	73	4 (5.5)	--	--
Fall (Sep-Nov)	252	19 (7.5)	NSS	
Winter (Dec-Feb)	161	6 (3.7)	NSS	
Spring (Mar-May)	206	9 (4.4)	NSS	
Training site				
Navy, Illinois*	222	5 (2.3)	--	--
Marines, California	3	0 (0.0)	NSS	
Marines, South Carolina	282	10 (3.6)	NSS	
Army, South Carolina	4	1 (25.0)	11.4	(1.1–116.3)
Army, Kentucky	23	4 (17.4)	6.6	(2.1–21.8)
Army, Missouri	37	7 (18.9)	6.7	(2.6–17.7)
Army, Oklahoma	19	2 (10.5)	NSS	
Air Force, Texas	102	9 (8.8)	2.7	(1.2–6.3)

NOTE. OR = odds ratio; CI = confidence interval; NSS = not statistically significant.
* Reference category for multivariable exact logistic regression.
[†]Variable not statistically significant at univariate level; not included in multivariable model.

Table 4. Factors associated with *emm* gene type of GAS isolates.

Variable	Total Isolates	1 *n* = 33 %	2 *n* = 50 %	29 *n* = 123 %	3 *n* = 104 %	44/61 *n* = 62 %	6 *n* = 92 %	75 *n* = 44 %	Other* *n* = 175 %	Univariate χ^2 p-value†
Gender										
Male	587	4.94	8.01	18.6	16.2	8.86	13.8	6.47	23.17	= *0.018*
Female	96	4.17	3.13	14.6	9.38	10.4	11.5	6.25	40.63	
Age, y										
17-18	196	1.02	8.16	20.9	20.4	5.61	10.2	5.61	28.06	*=0.002*
19-20	278	4.68	6.83	17.99	16.19	8.99	15.83	7.91	21.58	
21-22	98	4.08	6.12	18.37	11.22	13.27	13.27	6.12	27.55	
$\geq$ 23	107	11.2	8.41	12.2	7.48	12.2	14.02	4.67	29.91	
Season										
Summer (Jun-Aug)	73	4.11	2.74	27.4	6.85	2.74	8.22	6.85	41.10	< *0.001*
Fall (Sep-Nov)	250	3.6	12.4	25.6	23.6	5.20	2.80	0.80	26.00	
Winter (Dec-Feb)	159	6.92	3.77	16.4	10.1	13.8	21.4	9.43	18.24	
Spring (Mar-May)	204	5.88	5.39	6.37	11.8	12.3	22.1	10.8	25.49	
Training site										
Navy, Illinois	222	2.70	8.56	0.0	8.11	27.0	32.9	0.90	19.82	< *0.001*
Marines, California	3	66.7	33.3	0.0	0.0	0.0	0.0	0.0	0.0	
Marines, South Carolina	280	3.57	8.21	43.2	25.7	0.0	1.43	0.36	17.50	
Army, South Carolina	4	0.0	0.0	0.0	0.0	0.0	25.0	0.0	75.0	
Army, Kentucky	22	0.0	4.6	0.0	4.6	0.0	4.6	36.4	50.0	
Army, Missouri	36	2.78	8.33	5.56	30.6	5.56	2.78	5.56	38.89	
Army, Oklahoma	18	33.3	11.1	0.0	5.6	0.0	0.0	0.0	50.00	
Air Force, Texas	101	9.90	0.99	0.0	0.99	0.0	11.9	30.7	45.54	

*****Other category includes the following *emm* types: *emm*12 (*n* = 22), *emm*22 (*n* = 13), *emm*11 (*n* = 19), *emm5* (*n* = 14), *emm*89 (*n* = 15), *emm*28 (*n* = 19), *emm*18 (*n* = 10), *emm*27L/77 (*n*=14), *emm*4 (*n* = 9), *emm*58 (*n* = 3), *emm*73 (*n* = 6), *emm*82 (*n* = 2), *emm*96 (*n* = 3), *emm*9 (*n* = 6), and 20 additional types with *n* = 1.

†p-values based on Monte Carlo estimate for Pearson chi-square exact test

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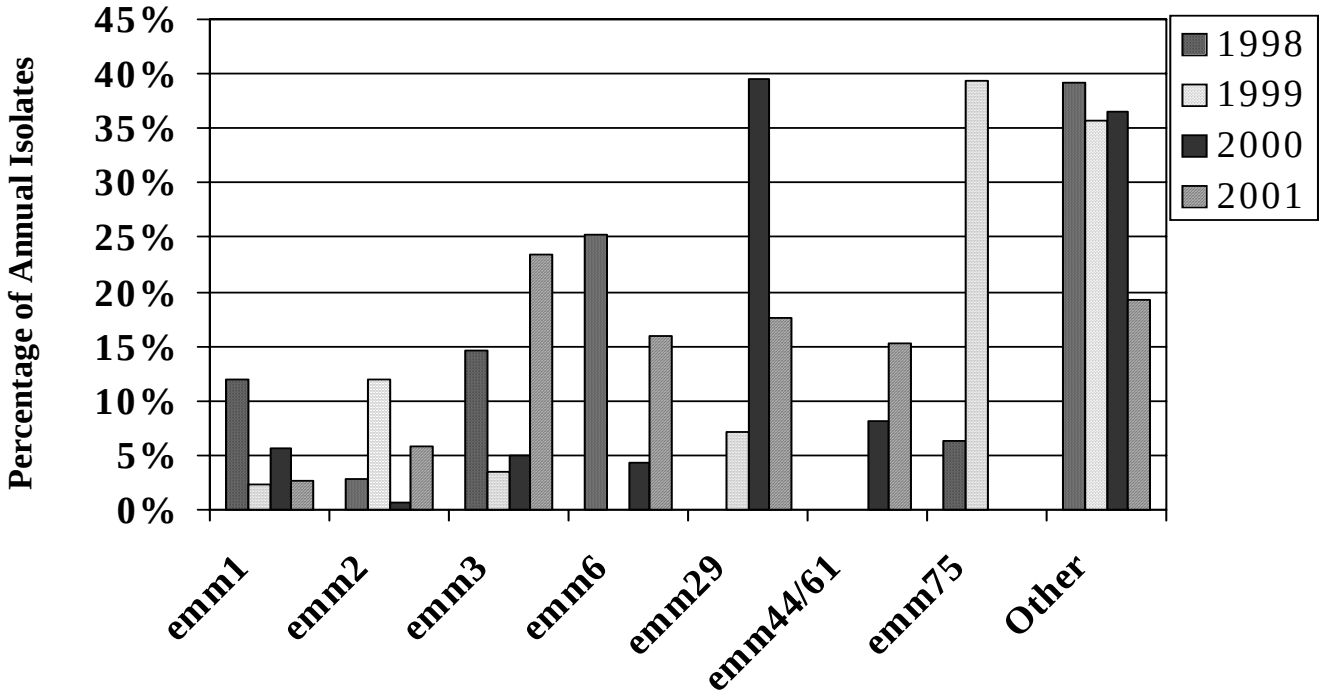
Figure 1. Most common *emm* types of GAS isolates identified in military basic training surveillance, 1998-2001.



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14. ABSTRACT (maximum 200 words)

Active surveillance for group A streptococci (GAS) was conducted among military trainees with pharyngitis at 8 U.S. military basic training sites between January 1998 and December 2001. Antibiotic resistance and *emm* gene type distribution were assessed for 692 GAS isolates. Antibiotic susceptibility testing revealed 100% sensitivity to penicillin, levofloxacin and vancomycin. Forty-four isolates (6.4%) were resistant to erythromycin, 38 (5.5%) resistant to tetracycline, 22 (3.2%) resistant to clindamycin, and 14 isolates (2.0%) showed multidrug resistance. The most prevalent genotypes were *emm29* (18.0%), *emm3* (15.2%), *emm6* (13.5%), *emm44/61* (9.1%), *emm2* (7.3%), *emm75* (6.4%), and *emm1* (4.8%). An association was found among distinct *emm* types and geographic location. Erythromycin resistance was strongly associated with *emm*75 and *emm*29 isolates ($p < 0.001$). Continued monitoring of antibiotic susceptibility and genetic epidemiology of GAS isolates is important for directing appropriate prevention and treatment strategies among U.S. military populations.

15. SUBJECT TERMS
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